

Appl. No. 09/546,857
Amdt. dated May 28, 2003
Reply to Office Action of April 28, 2003

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

Claims 1-19 (canceled)

A1
Claim 20 (new): A VEGF variant polypeptide comprising amino acid substitutions D63S, G65M, and L66R.

Claim 21 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 20.

Claim 22 (new): A vector comprising the nucleic acid of claim 21.

Claim 23 (new): A VEGF variant polypeptide of comprising one or more amino acid substitutions at residues 63 to 66 of native VEGF and one or more amino acid substitutions at amino acid residues 17 to 25 of native VEGF, wherein the amino acid substitutions increase the relative binding affinity ratio of KDR to FLT-1.

Claim 24 (new): The VEGF variant of claim 23 wherein the amino acid substitution(s) at amino acid residues 17 to 25 comprises one or more amino acid substitutions at amino acid residues 18, 21, 22, or 25 of native VEGF.

Claim 25 (new): The VEGF variant of claim 23, wherein the amino acid substitution(s) comprises D63S, G65M, or L66R.

Claim 26 (new): The VEGF variant of claim 25, wherein the amino acid substitution(s) further comprises M18E, Y21L, Q22R, or Y25S.

Claim 27 (new): The VEGF variant of claim 25, wherein the amino acid substitutions further comprise M18E, Y21L, Q22R, and Y25S.

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Claim 28 (new): The VEGF variant of claim 23 wherein the amino acid substitutions comprises D63S, G65M, and L66R

Claim 29 (new): The VEGF variant of claim 28, wherein the amino acid substitution(s) further comprises M18E, Y21L, Q22R, or Y25S.

Claim 30 (new): The VEGF variant of claim 28, wherein the amino acid substitutions further comprise M18E, Y21L, Q22R, and Y25S.

Claim 31 (new): A VEGF variant of claim 23, comprising one of the following combinations of amino acid substitutions:

- (a) M18E, D63S, G65M, and L66R;
- (b) Y21L, D63S, G65M, and L66R;
- (c) Q22R, D63S, G65M, and L66R;
- (d) Y25S, D63S, G65M, and L66R;
- (e) M18E, Y21L, D63S, G65M, and L66R;
- (f) M18E, Q22R, D63S, G65M, and L66R;
- (g) M18E, Y25S, D63S, G65M, and L66R;
- (h) Y21L, Q22R, D63S, G65M, and L66R;
- (i) Y21L, Y25S, D63S, G65M, and L66R;
- (j) Q22R, Y25S, D63S, G65M, and L66R;
- (k) M18E, Y21L, Q22R, D63S, G65M, and L66R;
- (l) M18E, Q22R, Y25S, D63S, G65M, and L66R;
- (m) Y21L, Q22R, Y25S, D63S, G65M, and L66R;
- (n) M18E, Y21L, Q22R, Y25S, and D63S;
- (o) M18E, Y21L, Q22R, Y25S, and G65M;
- (p) M18E, Y21L, Q22R, Y25S, and L66R;
- (q) M18E, Y21L, Q22R, Y25S, D63S, and G65M;
- (r) M18E, Y21L, Q22R, Y25S, D63S, and L66R;
- (s) M18E, Y21L, Q22R, Y25S, G65M, and L66R; or

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(t) M18E, Y21L, Q22R, Y25S, D63S, G65M, and L66R.

Claim 32 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 23.

Claim 33 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 27.

Claim 34 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 29.

Claim 35 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 31.

Claim 36 (new): A vector comprising the nucleic acid of claim 32.

Claim 37 (new): A host cell comprising the vector of claim 36.

Claim 38 (new): A composition comprising the VEGF variant of claim 23 and a carrier.

Claim 39 (new): The composition of claim 38, wherein the carrier is a pharmaceutically acceptable carrier.

Claim 40 (new): An assay for detecting KDR receptor, comprising contacting an isolated cell or tissue with a VEGF variant of claim 23 and assaying for binding of the VEGF variant to the cell or tissue.

Claim 41 (new): A method for stimulating phosphorylation of a KDR receptor, comprising contacting a cell with a VEGF variant of claim 23 in amount effective to stimulate phosphorylation of the KDR receptor.

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Claim 42 (new): A method for stimulating MAP kinase activation, comprising contacting a cell with a VEGF variant of claim 23 in amount effective to stimulate phosphorylation of MAP kinase.

Claim 43 (new): A method for stimulating PLC-gamma activation, comprising contacting a cell with a VEGF variant of claim 23 in amount effective to stimulate phosphorylation of PLC-gamma.

Claim 44 (new) A method for stimulating PI 3'-kinase activation, comprising contacting a cell with a VEGF variant of claim 23 in amount effective to stimulate phosphorylation of PI 3'-kinase.

Claim 45 (new): A method for stimulating vasculogenesis or angiogenesis, comprising contacting a cell expressing KDR receptor with an effective amount of a VEGF variant of claim 23.

Claim 46 (new): A method for promoting the migration of endothelial cells, comprising contacting endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim 23.

Claim 47 (new): A VEGF variant polypeptide comprising one or more amino acid substitutions at amino acid residues 17 to 25 of native VEGF, wherein the amino acid substitutions increase the relative binding affinity ratio of KDR to FLT-1.

Claim 48 (new): The VEGF variant of claim 47, wherein the amino acid substitution(s) comprises one or more amino acid substitutions at amino acid residues 18, 21, 22, or 25 of native VEGF.

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Claim 49 (new): The VEGF variant of claim 47, wherein the amino acid substitution(s) comprise M18E, Y21L, Q22R, or Y25S.

Claim 50 (new): The VEGF variant of claim 47, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

Claim 51 (new): The VEGF variant of claim 47, wherein the amino acid substitutions comprise F17I, M18E, Y21F, Q22K, and Y25S.

Claim 52 (new): The VEGF variant of claim 47, wherein the amino acid substitutions comprise F17I, M18E, Y21F, Q22E, and Y25I.

Claim 53 (new): An isolated nucleic acid sequence encoding the VEGF variant of claim 47.

Claim 54 (new): The isolated nucleic acid sequence of claim 6 encoding the VEGF variant of claim 50.
